REMARKS

Claims 1-21 were pending in the application. Claims 11-19 and 21 were withdrawn. Claims 1 and 2 have been amended in this response. Support for these amendments is found in the specification as filed. These amendments introduce no new matter. By the amendments, Applicant does not acquiesce to the propriety of any of the Examiner's rejections and does not disclaim any subject matter to which Applicant is entitled. *Cf. Warner Jenkinson Co. v. Hilton-Davis Chem. Co.*, 41 U.S.P.Q.2d 1865 (U.S. 1997).

I. Rejection under 35 U.S.C. § 112, second paragraph

Claims 1-10 and 20 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Office Action, page 3. The Examiner alleges that "the term 'an effective amount' in claim 1 is not clear." *Id.* Applicants respectfully traverse.

The test for definiteness under 35 U.S.C. § 112, second paragraph, is whether "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986). The Examiner points to *In re Frederiksen et al.* as supporting the indefiniteness of the term "an effective amount." 213 F.2d 547 (CCPA 1954). *Frederiksen* is authority for the proposition that the term "an effective amount" is indefinite when a claim fails to state the function to be achieved. *In the Matter of the Application of Charles Andrew Watson,* 517 F.2d 465, 477 (CCPA 1975). In *Frederiksen* the accused claim was indefinite because it completely "fail[ed] to state the function which [was] to be rendered effective." *Frederiksen* at 548. However, the function to be rendered effective in the present invention is clearly stated in the claim: "administering to a patient an effective amount of a cupredoxin . . . to promote cell death . . . ." Claim 1. *Frederiksen* is thus inapt for evaluating the indefiniteness of this claim.

More appropriate is *Watson,* wherein the accused claim was held not to be indefinite that recited "an effective amount of a germicide suitable for use in oral hygiene," because "germicide" indicated that germicidal action was the effect to be produced. *Watson* at 477. Likewise in claim 1 of the present invention, "to promote cell death" is the function to be effected by administration of cupredoxin, its functional variants or derivatives.

The Examiner admits that the specification "provide[s] a teaching for treating a disease condition or a cell with variant amounts of azurin" (Office Action, page 3), but

alleges that "one skilled in the art could not determine what 'an effective amount' is" because of the differing wild-type azurin cytoxicity results provided in Figures 12(b) and 14. *Id.* However, the Applicants would point out that the cytotoxicity results differ in Figures 12(b) and 14 because the cytotoxic effects of azurin are being tested on different cell types, that are obtained by different methods. Figure 12(b) pertains to cytotoxicity against macrophages, and derives from the data of Example 20 wherein macrophages were derived according to the process described in Example 2. Paragraph [0167], page 50. Figure 14 pertains to cytotoxicity against melanoma cells, and derives from the data of Example 22 wherein UISO-Mel-2 cells were obtained according to another process as described in Example 13. Paragraph [0173], page 52. Applicants respectfully submit that one skilled in the art would expect azurin to effect variant levels of cytotoxicity depending on the types of cells targeted; and further, that cells derived via different methods may also exhibit somewhat variant resistance to cytotoxicity. Based on the teachings of the present invention, one skilled in the art would know that 100ug/ml azurin is effectively cytotoxic against over 50% of macrophage cells under these conditions (Fig. 12(a)), and that it would require approximately 400ug/ml azurin to effectuate cytotoxicity of 40% of melanoma cells, under these conditions (Fig. 14).

Because claim 1 clearly states that the function to be effected by administering cupredoxin (and its functional variants or derivatives) is the promotion of cell death, and the Applicants have demonstrated the levels of azurin needed for cytotoxicity in macrophages and melanoma cells, the Applicants submit that "an effective amount" is not indefinite. One skilled in the art "will be able to determine from the disclosure, including the examples, what an effective amount [for promoting cell death] is." *Watson* at 477. Accordingly, Applicant respectfully requests that this rejection of claims 1-10 and 20 under 35 U.S.C. § 112, second paragraph be reconsidered and withdrawn.

II. Rejection under 35 U.S.C. § 112, first paragraph

A. Written description

Claims 1-10 and 20 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Office Action, page 4. Specifically, the Examiner states that "only a method of treating a condition related to resistance to cell death comprising administering azurin consisting of SEQ ID NO: 1, but not the full breadth of the claims, meets the written description provision. . . ." Office Action, page 6. Claims 1 and 2 are amended in this response. Applicants respectfully traverse the rejection of claims 1-10

and 20, and request that the Examiner revisit the written description issue in light of the present claim amendments and Applicants' explanations below.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. *See, e.g., Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003). An applicant complies with the written description requirement "by describing the invention, with all its claimed limitations, not that which make it obvious," and by using "such descriptive means as words, structures, figures, diagrams, formulas, etc. that set forth the claimed invention". *Regents of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1666 (Fed. Cir. 1997). The test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter, rather than the presence or absence of literal support in the specification for the claim language. *In re Kaslow*, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983).

The Examiner alleges that only the administration of "azurin consisting of SEQ ID NO: 1" for treating a condition related to cell death resistance meets the written description provision of 35 U.S.C. § 112, first paragraph. Office Action, page 6. Applicants interpret this to mean the Examiner alleges that the administration of plastocyanin, and/or the plastocyanin comprising the amino acid of SEQ ID NO: 2, does not meet the written description requirement. Applicants respectfully submit that plastocyanin, like azurin, is a cupredoxin. Paragraph [072], page 18. The specification of the present invention teaches the use of plastocyanin as a cytotoxic factor: the cytotoxic activity of plastocyanin derived from *Phormidium laminosum* is demonstrated in Example 23 (paragraph [0175], page 52) and Fig. 15. The amino acid sequence of this plastocyanin from *P. laminosum* is fully disclosed in the specification as SEQ ID NO: 2. Paragraph [072], page 17. The three-dimensional structures of various plastocyanins are known to be conserved. Paragraph [073], page 17. Applicants therefore respectfully submit that the administration of a plastocyanin, and further, the plastocyanin comprising SEQ ID NO: 2, meets the written description requirement of 35 U.S.C. § 112, first paragraph.

Second, the Examiner alleges that the specification does not teach a method of "administering any plastocyanin comprising mutants or any mutants of variants of azurin . . . ." Office Action, page 4. As no claims are made herein to the administration of plastocyanin

comprising mutants of azurin, Applicants do not understand the allegation and respectfully request clarification from the Examiner.

The Examiner alleges that the specification does not teach "a method of treating resistant cells with mutants or variants of plastocyanin comprising the polypeptide [of] at least 90% identity to SEQ ID NO: 2." Office Action, page 4. Claims 1 and 2 have been amended to claim the administration of "a cupredoxin, or a functional variant or derivative thereof . . ." to promote cell death. This amendment is supported in the specification, wherein is taught the creation of variant factors still retaining cytotoxic functionality: "[c]ytoxic factors also can be . . . modified . . . to produce variants that lack an ATP-utilizing enzyme or redox activity, but retain toxicity" (paragraph [0112], page 31); "[m]utations and/or truncations of cytotoxic factors can produce cytotoxic agents . . . also demonstrating functional activity" (*id.*); "[s]uch modified or altered cytotoxic factors also are included in the scope of the present invention" (*id.*).

The amino acid sequences for several variants are disclosed, with description as to how to create these variants. Fig. 11; Example 19, paragraph [0159], page 47 *et seq.* Also described is a test to select for functional variants. Example 20, paragraph [0167], page 50 *et seq.* Finally, evidence for the cytotoxic activity of the variants is presented in Fig. 12(b). Applicants respectfully submit that the specification does teach the administration of functional mutants or variants of cupredoxins to treat resistance to cell death: by amending the claims to recite functional variants of a cupredoxin, describing methods of creating variants, describing methods for selecting functional variants, and teaching variant sequences based on these methods that successfully exhibit cytotoxic effects.

Further, the specification teaches the creation of derivatives or analogs with amino acid sequences that have a range of 65-99% identity with the sequences of cytotoxic factors. Paragraph [0114], page 31. Therefore, the specification provides written description for a cupredoxin or functional variant with an amino acid sequence with at least 90% sequence identity with SEQ ID NO: 1 or 2.

The Examiner alleges that the specification "does not teach . . . either plastocyanin or azurin or their mutants or variants [which] binds to p53 in the process of inducing cell death or cytotoxicity." Office Action, page 4. Applicants submit that the specification does indeed teach the induction of cell death, specifically via apoptosis, in the binding of a cupredoxin or its functional variants to p53. Paragraph [081], page 22 teaches that "[a]zurin forms a complex with p53, stabilizes it . . . thereby inducing apoptosis . . . ." (*citing* T. Yamada *et al., Infec. Immun.* 70:7054-7062 (2002)). Further, whether mutant azurin proteins exhibit

cytotoxic activity depends on whether there is continued ability of the mutant to form p53 complexes. Paragraphs [083]-[086], pages 22-23. After describing the association of cytotoxicity with cupredoxin binding to p53, Applicants demonstrate this fact in Example 17, wherein cells lacking expression of p53 (i.e., cells of a p53(-/-) breast cell line) required twice as much azurin to achieve a level of cell death commensurate with that of the cells fully expressing p53 (i.e., cells of a p53(+/+) breast cell line), in the same amount of time. Paragraphs [0149]-[0151], page 45.

The Examiner states that one "cannot envision the detailed chemical structure(s) and functional attribute(s) of the encompassed genus of variants . . . of azurin and plastocyanin . . . ." and cites *Fiddes* as authority for unpatentability of a class due to lack of written description. Office Action, page 6. Claims in *Fiddes* to a "DNA molecule consisting essentially of a DNA sequence encoding mammalian basic fibroblast growth factor [FGF]" were unpatentable due to lack of written description. *Fiddes v. Baird,* 30 USPQ2d 1481, 1483 (BPAI, 1993). The patent in *Fiddes* claimed DNA to the broad class of mammalian FGFs, but taught only the amino acid sequence for bovine pituitary FGF. *Id.* No FGF DNA was taught in the patent, but for a theoretical sequence based on the amino acid sequence. *Id.* As the Board held, "[a]n adequate description of a DNA requires more than a mere statement that it is part of the invention"; "what is required is a description of the DNA itself." *Id.* For evidence of conception of a DNA sequence the Board required the specification to present its "structure, formula, chemical name, or physical properties." *Id.*

The patent of *Fiddes* is distinguished from the present invention in that the Applicants herein do provide written description of the azurin and plastocyanin amino acids claimed (SEQ ID NOS: 1 and 2, respectively). Claim 1 as amended is directed to functional variants of cytotoxic factors. The amino acid sequences for several variants are provided, with written description as to how to create these variants (Fig. 11; Example 19, paragraph [0159], page 47 *et seq.*) and how to screen for cytotoxic functionality (Example 20, paragraph [0167], page 50 *et seq.*). Finally, evidence for the cytotoxic activity of the variants is presented in Fig. 12(b).

Accordingly, Applicant respectfully requests that this rejection of claims 1-10 and 20 under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn.

B. Enablement

Claims 1-10 and 20 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. Office Action, page 6. Specifically, the Examiner states that the claims contain subject matter "which was not described in the specification in such a way as to enable one skilled in the art . . . to make and/or use the invention." *Id.* Claims 1 and 2 are amended in this response. Applicants respectfully traverse the rejection of claims 1-10 and 20.

The examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (C.C.P.A 1971). As stated by the court, "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." 439 F.2d at 224, 169 USPQ at 370.

The Examiner bases the present 35 U.S.C. § 112, first paragraph rejections for alleged lack of enablement on the Examiner's application of the *In re Wands* factors (858 F.2d 731, 737 (Fed. Cir. 1988); MPEP § 2164.01(a)) to the present invention. Office Action, page 6. Specifically, the Examiner alleges that there is a "lack of guidance, lack of examples, and lack of predictability" associated with the claimed methods of treating conditions related to cell death by administering azurin, plastocyanin or variants thereof, and that one skilled in the art "would be forced into [undue] experimentation" to practice the claimed invention. Applicants respectfully traverse, and request the Examiner revisit the enablement issue in light of the present claim amendments and the Applicants' explanations concerning the *In re Wands* factors provided below.

Claims 1 has been amended to claim a method of treating a condition related to cell death resistance by "administering to a patient . . . a cupredoxin, or a functional variant or derivative thereof . . ." to promote cell death. This amendment is supported in the

specification, wherein is taught the creation of variant factors still retaining cytotoxic functionality: "[c]ytoxic factors also can be . . . modified . . . to produce variants that lack an ATP-utilizing enzyme or redox activity, but retain toxicity" (paragraph [0112], page 31); "[m]utations and/or truncations of cytotoxic factors can produce cytotoxic agents . . . also demonstrating functional activity" (*id.*); "[s]uch modified or altered cytotoxic factors also are included in the scope of the present invention" (*id.*). As to administration to a patient, the specification teaches a cytotoxic factor "optionally incorporated in a pharmaceutical carrier" (paragraph [009], page 4); e.g., "incorporated into a pharmaceutical composition for use in the prevention and treatment of conditions related to abnormal cell proliferation" (paragraph [010], page 4).

The Examiner alleges that the specification of the present invention fails to teach "a method of treating resistant cells with mutants or variants of plastocyanin comprising the polypeptide [with] at least 90% identity to SEQ ID NO: 2 . . . ." Office Action, page 7. The specification does provide guidance and working examples in the use of plastocyanin as a cytotoxic factor: preparation of plastocyanin and analysis of its cytotoxic activity is taught in Example 23, using the same methods as for azurin, via standard protocols well-known to those skilled in the art (paragraph [0175], page 52) and Fig. 15.

Guidance and working examples providing methods for creating variants of azurin are described in the specification. Beginning on page 31 of the specification is a discussion of the modification of cytotoxic factors to create functional variants, of varying percent amino acid sequence identity with cytotoxic factors. Paragraph [0112] *et seq.* Preferred amino acid substitutions and classes are taught in Tables 1 and 2. Pages 32 and 33. Example 19 teaches the preparation of azurin mutants by site-directed mutagenesis or by the creation of chimeras, using standard laboratory protocols well known to those of skill in the art. Paragraph [0159], page 47 *et seq.* These methods of creating azurin mutants are also applicable to the creation of variants of plastocyanin or any other cupredoxin. Cytotoxic assays to screen for functional mutants are also provided and easily understandable by one of skill in the art. Example 20, paragraph [0167], page 50 *et seq.*

The Examiner alleges that the specification does not teach a method of "administering any plastocyanin comprising mutants or any mutants of variants of azurin . . . ." Office Action, page 7. As no claims are made herein to the administration of plastocyanin comprising mutants of azurin, Applicants do not understand the allegation and respectfully request clarification from the Examiner.

The Examiner alleges that the specification does not teach "a method of treating resistant cells with mutants or variants of plastocyanin comprising the polypeptide [of] at least 90% identity to SEQ ID NO: 2." Office Action, page 4. The amino acid sequences for several variants are taught herein, with description as to how to create these variants. Fig. 11; Example 19, paragraph [0159], page 47 *et seq.* Also described is a test to select for functional variants. Example 20, paragraph [0167], page 50 *et seq.* Finally, evidence for the cytotoxic activity of the variants is presented in Fig. 12(b). Applicants respectfully submit that the specification does provide guidance in the administration of functional mutants or variants of cupredoxins to treat resistance to cell death.

Further, the specification teaches the creation of derivatives or analogs with amino acid sequences that have a range of 65-99% identity with the sequences of cytotoxic factors. Paragraph [0114], page 31.

The Examiner alleges that the specification "fails to provide objective evidence, which azurin or plastocyanin or variants . . . binds to p53 to promote cell death" Office Action, page 7. Applicants submit that the specification does indeed teach the induction of cell death, specifically via apoptosis, in the binding of a cupredoxin or its functional variants to p53. Paragraph [081], page 22 teaches that "[a]zurin forms a complex with p53, stabilizes it . . . thereby inducing apoptosis . . . ." (*citing* T. Yamada *et al., Infec. Immun.* 70:7054-7062 (2002)). Further, whether mutant azurin proteins exhibit cytotoxic activity depends on whether there is continued ability of the mutant to form p53 complexes. Paragraphs [083]-[086], pages 22-23. After describing the association of cytotoxicity with cupredoxin binding to p53, Applicants demonstrate this fact in Example 17, wherein cells lacking expression of p53 (i.e., cells of a p53(-/-) breast cell line) required twice as much azurin to achieve a level of cell death commensurate with that of the cells fully expressing p53 (i.e., cells of a p53(+/+) breast cell line), in the same amount of time. Paragraphs [0149]-[0151], page 45.

Additionally, the Examiner alleges that the specification teaches away from the claimed method of treating cells with azurin or plastocyanin mutants to promote cell death, and points to Examples 20 and 21, Figures 12 and 13 as demonstrating that the mutants "M44KM64E (SEQ ID NO: 7), C112D (SEQ ID NO: 6), or S3S5 etc. do not have cytotoxicity to the cells and no apoptosis induction . . . ." Office Action, page 7. Applicant respectfully submits that this is an incorrect summation of the data provided. As stated in the specification, the single mutant C112D "shows significant cytotoxicity" (paragraph [083], page 22), and indeed Figure 12(a) demonstrates this mutant's cytotoxic activity: 100ug/ml C112D azurin effected cell death in approximately 30% of treated cells, compared to

approximately 50% of cells treated with wild type azurin. C112D also has pronounced apoptotic activity. Figure 13 demonstrates the apoptotic activity of this mutant: at 100 ug/ml the C112D azurin demonstrated a higher level of apoptotic cytotoxicity than wild type.

The double mutant M44KM64E does not have the same cytotoxic activity as, and less apoptotic activity than the single mutant C112D, as shown in Figures 12(a) and 13. However, the claims of the present invention are drawn to administering cupredoxins and/or variants or derivatives "to promote cell death." Claim 1. Throughout the specification the promotion of cell death by cytotoxic factors is described as being effected *not only* by apoptosis but also by growth arrest: "'cytotoxic factor' refers to a . . . factor which acts to cause cell death or cellular growth arrest" (paragraph [032], page 8); "[t]he present invention provides cytotoxic factors that . . . stimulate cell death by necrosis or apoptosis or that cause cellular growth arrest" (paragraph [043], page 10); "cytotoxic factors induce apoptosis or cellular growth arrest in cancer cells" (paragraph [046], page 11); etc. Figures 12 and 13 are demonstrations of cytotoxicity and apoptosis only, not cell growth arrest. As stated in the specification, M44KM64E does show significant inhibition of cell cycle progression (paragraph [086], page 23; and paragraph [0119], page 34). For this reason M44KM64E would be desirable as a variant cupredoxin useful in promoting cell death. Indeed, the specification teaches "combinations of cytotoxic factors such as azurin and C_{551} (or M44KM64E) can achieve more effective inhibition of tumor progression by inhibiting both apoptosis and growth arrest." Paragraph [087], page 24. Whether through apoptosis or the inhibition of cell growth, the promotion of cell death is desirable by any means.

For the foregoing reasons, and following amendment of the claims, Applicants respectfully request that the rejection of claims 1-10 and 20 under 35 U.S.C. 112, first paragraph, for lack of enablement be reconsidered and withdrawn.

III. Rejection under 35 U.S.C. § 102

Claims 1-6 are rejected under 35 U.S.C. § 102(a) as being anticipated by Zaborina et al. (*Microbiology*, vol. 146, pages 2521-2530, Oct. 2000); ("Zaborina")). Office Action, p. 8. Claim 1 is amended in this response. Applicant respectfully traverses the rejection of claims 1-6.

In order to support an anticipation rejection under 35 U.S.C. § 102, the Examiner must illustrate that each and every element of a claimed invention was disclosed within a single prior art reference. *In re Bond*, 15 U.S.P.Q.2d 1566, 1567 (Fed. Cir. 1990). A claimed invention is anticipated only when it is "known to the art in the detail of the claim." *Karsten*

Manufacturing Corp. v. Cleveland Golf Co., 242 F.3d 1376, 1383 (Fed. Cir. 2001). In other words, not only must the limitations of the claim be shown in a single prior art reference, the limitations must be "arranged as in the claim." *Id.* Zaborina does not teach or disclose every element of the claimed invention.

The Examiner interprets the claims as "administering [a cytotoxic factor] to a cell to promote cell death." Office Action, page 9. Claim 1 is amended in this response to recite "[a] method of treating a condition related to resistance to cell death, comprising administering to a patient an effective amount of a cupredoxin . . . ." Support for the amendment is found in the specification as filed. The specification teaches a cytotoxic factor "optionally incorporated in a pharmaceutical carrier" (paragraph [009], page 4); e.g., "incorporated into a pharmaceutical composition for use in the prevention and treatment of conditions related to abnormal cell proliferation" (paragraph [010], page 4). Zaborina relates to methods of treating conditions related to cell death "by contacting the resistant cell with [an] effective amount of azurin . . . ." Office Action, page 9. For this reason Zaborina does not teach or disclose every element of the claimed invention.

Accordingly, Applicant respectfully requests that this rejection of claims 1-6 under 35 U.S.C. § 102(a) be reconsidered and withdrawn.

IV. Double patenting

Claims 1, 3 and 20 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting, as allegedly being unpatentable over claims 18, 20 and 21 of co-pending Application No. 11/435,592. Office Action, page 10. Applicant respectfully traverses.

The Examiner has instructed that a terminal disclaimer in compliance with 37 C.F.R. § 1.321(c) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting ground. Without addressing the propriety of the Examiner's rejection, and specifically the Examiner's interpretation of what the cited references teach or suggest, Applicants respectfully and properly defer addressing the present rejection until there is allowable subject matter in the present application. At that time, a terminal disclaimer will be filed if warranted by the Examiner's rejection in view of the allowed claims.

V. Conclusion

Applicant has properly and fully addressed each of the Examiner's grounds for rejection. Applicant submits that the present application is now in condition for allowance. If the Examiner has any questions or believes further discussion will aid examination and advance prosecution of the application, a telephone call to the undersigned is invited. If there are any additional fees due in connection with the filing of this amendment, please charge the fees to undersigned's Deposit Account No. 19-1853. If any extensions or fees are not accounted for, such extension is requested and the associated fee should be charged to our deposit account.

Respectfully Submitted,

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